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CYTOLOGY OF CHLOROPHYLL TYPES OF MAIZE

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(WITH PLATES XI-XVI)

Introduction

During recent years there have been discovered a number of instances of chlorophyll inheritance which are characterized by features due to unusual modes of chlorophyll distribution and behavior. These phenomena are obviously of interest both to the geneticist and the cytologist; to the geneticist because some of them almost certainly represent distinct categories of inheritance, and to the cytologist because of the manner in which they involve the origin and behavior of cell organs other than the nucleus, which latter has been generally held to be mainly responsible for the transmission and development of inherited characters. Much attention has been devoted to this subject by geneticists, whereas the cytological aspects of the problem have not as yet received as much attention. It has been felt that a more adequate knowledge of the behavior of the visible constituents of the cell is of prime importance in the attempt to find a solution of the problem. The present study was undertaken for the purpose of determining whether or not there are in the cells of certain chlorophyll types of maize any visible structural differences which can be shown to be responsible for, or correlated with, the known genetic behavior of these plants.

TYPES OF PLASTID INHERITANCE

There have been described numerous cases of chlorophyll variation which involve an unequal and frequently a very irregular distribution of green color in various regions of the plant. The inheritance of such color patterns has not been found always to conform to the behavior usually ascribed to Mendelian characters. A number of these cases will be reviewed briefly, and for convenience those which have been reported in maize and other known cases will be considered separately.

PLANTS OTHER THAN MAIZE.—A large number of more or less distinct color patterns have been shown to behave as simple Mendelian recessives. Albino seedlings, devoid of chlorophyll and which consequently die in the seedling stage, have been reported in *Antirrhinum latifolium* and *Melandrium album* by BAUR (3), in *Hordeum distichum* by KIESSLING (38), and in *Phaseolus vulgaris* by TJEBBES and KOOIMAN (55). Pale green seedlings have been described in *Urtica pilulifera* by CORRENS (6), and in *Ipomoea hederacea* by MIYAZAWA (49). Yellowish green seedlings have been reported in *Mirabilis jalapa xantha* by CORRENS (7), and in *Nicotiana rustica* by ALLARD (1). Various types of chlorophyll variegation have also been shown to be inherited as simple Mendelian recessives: in *Aquilegia vulgaris* by BAUR (3), in *Pisum arvense* by KAJANUS (37), and in *Capsella bursa-pastoris* and *Arabis albida* by CORRENS (8).

In other cases similar characters do not seem to be inherited in a Mendelian fashion, but are transmitted from one generation to the next through the female parent alone. Since the male parent does not seem to be definitely concerned in the transmission of the character, such cases have been called "maternal inheritance." The first case of this sort was described by CORRENS (6) in *Mirabilis jalapa albomaculata*. Plants of this strain produce branches having green leaves, others having white leaves, and still others with leaves which are partly green and partly white. All types of branches occur on the same plant, and all bear flowers. It was found that flowers from a green branch when self-fertilized produced only green seedlings in the following generation, and bred true thereafter. When crosses were made between flowers of green

and white branches occurring on the same plant, the resulting progeny always resembled the branch which produced the female gamete, regardless of the way in which the cross was made. The results obtained showed clearly that, so far as chlorophyll characters were concerned, the offspring were not affected by the pollen. CORRENS explained these results by assuming that the absence of chlorophyll was due to a cytoplasmic disease, which, although manifesting itself in the plastids, may or may not be limited to these organs. The diseased condition is accordingly transmitted from one generation to the next only through the egg cytoplasm, the male parent not affecting the character of the offspring, since no male cytoplasm is brought into the egg at the time of fertilization.

A situation similar to this was described by BAUR (3) in *Antirrhinum majus albomaculata* and *Aquilegia vulgaris*, and also by GREGORY (23) in *Primula sinensis*. These workers, however, are inclined to the view that two kinds of plastids, diseased and normal ones, become segregated during somatic mitoses to different cells and consequently to different regions of the plant tissue. This results in the variegated appearance common to plants of these strains. The diseased plastids are described by GREGORY (23, *pl. 10, fig. 10*) as being pale yellow and smaller than the normal plastids. In young actively growing leaf tissue both kinds are present in the same cell. Another interpretation should be placed on these figures of GREGORY, as will be discussed later.

A somewhat different case is that reported by BAUR (2) in *Pelargonium zonale albomarginata*. In this form plants occur which have green branches and entirely white branches. Flowers borne on either green or white branches when self-fertilized produce offspring in succeeding generations which are like the original branch. When, however, crosses are made between green and white branches, mosaic seedlings (green and white) result, regardless of the way in which the cross is made. This case differs from that of *Mirabilis* in that the inheritance is not the maternal type. Here both the male and female gametes must be concerned in the transmission of the character. BAUR is led to assume that plastids rather than the nucleus are directly responsible for this unusual type of inheritance, and that they are brought in by the male

gamete to the cytoplasm of the egg at the time of fertilization. This assumption is directly contradictory to that of CORRENS. BAUR further assumes that there are present in these plants two kinds of plastids, green and colorless ones, which are permanent cell organs and are sorted out and unequally distributed to daughter cells during somatic mitoses. Thus there results a segregation of green and colorless plastids in different parts of the plant, and this is held to account for the absence of chlorophyll in certain regions of the plant. BAUR (4) later reported similar cases in a strain of *Antirrhinum majus albomaculatum* and in *Aquilegia vulgaris*.

Additional evidence has been furnished by IKENO (36), who has worked with variegated races of *Capsicum annuum* and obtained results similar to those of BAUR in *Pelargonium*. Such strains of *Capsicum*, however, differ from *Pelargonium zonale albomarginata* in that all the plants produced show some degree of variegation, although green branches may occur. Furthermore, when pollen is taken from flowers on either variegated or green branches of a variegated strain and used to pollinate flowers on normal green plants, the resulting progeny are always variegated, although to a less degree than in the variegated parents. IKENO believes that this character is not controlled by the nucleus, but by plastids (diseased or normal) which are transmitted from one generation to the next by both parents. An apparently significant fact in this connection is that the two types of hereditary transmission, maternal and biparental, have not been found to occur in the same species. It is possible that this may mean that male cytoplasm regularly enters the egg in some species and not in others; but adequate cytological evidence for this is lacking.

MAIZE.—Numerous cases of chlorophyll inheritance have been reported in maize. Many distinct types have been described which differ greatly, not only in the mode of their inheritance, but also in the amount of chlorophyll present and the distribution and appearance of the pigment during the growth period. EMERSON (20) described several chlorophyll types, and presented evidence to show that albino seedlings conform to a Mendelian type of inheritance, and that the factor concerned is a simple Mendelian recessive. GERNERT (22) presented similar evidence. MILES (47)

furnished additional data on the inheritance of certain types of albinism, and as a result of a cytological investigation of the albino seedlings concluded that plastids are entirely absent in plants of that type. LINDSTROM (43), in a comprehensive study of chlorophyll inheritance in maize, described the behavior of eight distinct types, including those already reported on. In every case the inheritance was shown to be Mendelian. Two of these types, albino and yellow, are seedling characters, the plants failing to mature because of the absence of a sufficient amount of chlorophyll. In a third type, known as "virescent," the seedlings at first are yellowish white, but later become green, and in the mature condition cannot be distinguished from normal green plants. The five other characters described by LINDSTROM as "golden," "greenish-striped," "japonica white striped," "japonica yellow striped," and "fine striped," are manifested only in the mature plants; the young seedlings have a wholly normal appearance. From these studies LINDSTROM concluded that plastid inheritance in maize is typically Mendelian.

At the present time, however, cases are known in which the inheritance of certain aberrant chlorophyll types is not Mendelian. ANDERSON, in genetic studies as yet unpublished, has found that in a certain strain of maize there are produced some plants which are uniformly green, others uniformly yellowish green without sufficient chlorophyll to reach maturity, and still others with distinct yellowish green and green stripes.¹ Breeding experiments so far carried out have failed to show any inheritance of the unusual character through the male parent. The plants which are yellowish green lack a sufficient amount of chlorophyll to reach maturity, and no offspring have been obtained from them. The striped plants, either when self-fertilized or when pollen is used from a green plant of an unaffected strain, produce some green plants, some yellowish green plants, and some striped plants in varying proportions, which seemingly depend upon the amount of yellowish green tissue in the region of the plant producing the ear. Ears have been obtained which have produced only yellowish green

¹ Described in a paper read by E. G. ANDERSON before the Society of American Naturalists at Chicago, December, 1920. A published account is to appear shortly.

plants. The evidence indicates that this is a case of maternal inheritance. In no case has pollen from the affected plants of this strain produced any visible effect when used in crosses on green plants, either in the first or succeeding generations.

It is clearly evident from these cases that the inheritance of chlorophyll variations can hardly be accounted for on the basis of a single explanation. There are widely different categories which at present seem to be entirely distinct. In the case of the Mendelian behavior, the explanations offered are the same as for any other character behaving in a similar manner, although other cytoplasmic organs having a certain degree of individuality are apparently more directly involved in bringing the characters to expression. In this category characters expressed by intracellular organs are apparently under the control of nuclear factors, just as are other Mendelian characters expressed by tissues and multicellular organs. The hypotheses advanced to account for the non-Mendelian behavior of similar characters assume that the plastids themselves are permanent cell organs capable of transmitting certain characteristics, and are not controlled by nuclear factors; and furthermore, that in certain cases plastids are transmitted to succeeding generations by both parents, and in other cases only by the female parent. It must be admitted that such explanations are highly speculative, in view of the number of cytological observations which have so far been made.

METHODS AND TECHNIQUE

The general procedure followed has been to examine the material in the living condition, the observations thus made being supplemented by a study of fixed and stained preparations. It was found that meristematic tissue, including apical meristems and young leaf tips of germinating seeds, could readily be studied in this way. It was necessary in the case of older tissues to remove first the epidermal layer of cells before examining the mesophyll cells beneath. A solution of cane sugar of 7.5-10 per cent concentration was found to be a favorable medium in which to examine fresh material. Cells which are protected by one or two cell layers or by epidermal cells will remain living for a considerable period of

time, even in a water mount. Thus it was found that bits of embryonic (meristematic) tissue will remain living and apparently in an entirely normal condition for 24-36 hours in an isotonic solution of cane sugar. In most cases, however, observations were made immediately after making the mounts in order to avoid the possibility of any modification of the cell contents. Intra-vitam stains, such as neutral red, Cresyl blue, Janus green B, etc., have been used, but unexpectedly proved to be of relatively little value. Various methods of fixation and staining were used, including those of BENDA, REGAUD, CHAMPY, LAGUESSE, BENSLEY, and other special methods which have been reported as being useful in such studies.

Description

The chlorophyll types here described do not include all the known cases of chlorophyll abnormality in maize, but have been selected as representative of certain distinct categories, illustrating markedly different kinds of behavior, both in development and in inheritance. These types are discussed in genetical literature as "normal green," "Mendelian white," and "Mendelian virescent." The "maternal inheritance strain," discovered by ANDERSON, has not yet been described in the literature.

The development of plastids may readily be followed in the meristematic and mesophyll tissues of young rapidly growing seedlings. In the growth stages immediately following the germination of the seeds, leaf tissue is being formed through the activity of the apical stem meristem, and the embryonic leaves thus formed are increasing rapidly in size through an active division of the cells making up the leaf meristem and through a division and growth of the cells throughout the leaf tissue. The meristematic tissues during these and later stages furnish very favorable material for a study of the cytoplasmic inclusions of the living cells. Since it has been found that the cytoplasmic inclusions of the epidermal cells, as well as those of the vascular system, differ markedly from those of the mesophyll cells in mature leaf tissue, the study of plastid development has been limited to the regions from which mesophyll cells are derived.

NORMAL GREEN STRAIN

Detailed attention will be given to the development of plastids in a normal green plant, and in the consideration of the other types emphasis will be placed on the points in which such types differ from the normal plants.

APPEARANCE IN EMBRYONIC CELLS.—The youngest stages examined were those found in the cells of the promeristematic region of seedlings 36–48 hours after germination. This region occupies the apex of the stem, and is composed of undifferentiated parenchymatous cells which are actively undergoing division in all planes. In undifferentiated tissue in all cases the observations were made on subepidermal cells. In such cells the cytoplasm contains minute granules which may clearly be seen without the aid of special staining methods (fig. 1). These granules appear in the living cell as refringent globules, which are constantly changing their position in the cell as a result of cytoplasmic streaming; this movement seems to be a constant and characteristic feature of actively dividing embryonic cells. The size of these bodies is variable. In the promeristematic region they are rarely more than $1\ \mu$ in diameter, and careful observation reveals a closely intergrading series between these and other smaller ones lying just within the lower limit of visibility. Whatever their size, these bodies always appear sharply distinct from the homogeneous cytoplasm in which they are imbedded, and in this material cannot be interpreted as granular constituents of the cytoplasm itself. At this early stage the cytoplasm appears to be entirely free from other accumulations which might be confused with these small bodies.

Appearances which might be interpreted as division stages are frequently seen, both in the living cells and in fixed and stained preparations. In the living cells a condition which strongly suggests division by a simple constriction and bipartition of these bodies is frequently noted, but their extremely minute size and their constant movement make it impossible to follow a single granule for a sufficient length of time to obtain direct evidence for its division. Moreover, chance association and separation are of frequent occurrence, especially in cells displaying active cyto-

plasmic streaming. Association may be but momentary, or granules which have been seen to come together may remain attached for a considerable period of time. Thus when a separation is seen to occur, it is not possible to tell whether one is observing an actual division of a single granule, or a reseparation of two granules formerly distinct. The fact that a paired arrangement occurs much more frequently than do groups of three or groups of four might be considered as suggestive, but in view of the observed behavior described, this can scarcely be considered as convincing proof of division. This point is of interest in view of the undoubted division of similar bodies which have become larger in older cells. The marked variation in the size of these bodies and the fact that evidence for their division in these early stages is uncertain raise the question of their nature, a question which will be taken up in the discussion.

Observations on the living cells afford no evidence for the existence of more than one kind of body in the class of cell elements under discussion. An appearance suggesting the presence of two kinds of bodies, lighter and darker ones, is seen when the cells are examined with lenses of high magnification. This, however, is due to their relative position in the cytoplasm. In the living cells these minute bodies are continually in motion, and as they pass into the plane of focus first appear faintly colored, but when sharply in focus they are much darker in color, then become lighter again as they pass out of focus. Thus a single granule may appear light or dark depending on its position in the cytoplasm. The use of intra-vitam stains, such as are commonly employed in studies of cytoplasmic organization, and the fixing and staining methods which have been shown by recent workers to preserve the cytoplasm faithfully with its various inclusions, have also failed to differentiate distinct kinds. Treatment with osmic acid does not appreciably alter the appearance of these bodies (fig. 52). When treated with BENDA'S fixation and stained with haematoxylin, the cytoplasmic inclusions are well preserved, and their appearance is similar to that of living cells (fig. 53). Since these bodies have been found to occur as a constant feature of the cytoplasm of meristematic cells in maize, and inasmuch as they have been found to be definitely

concerned with the formation of chloroplasts, the term "proplastid" will be used for such bodies. Other cytoplasmic granules and transitory accumulations not concerned with the development of plastids may be present later. Oil globules which can be distinguished from the proplastids by their characteristic highly refractive appearance and microchemical reaction, and other metaplasmic masses in the vacuole (fig. 5) are to be found at certain stages. The chemical nature and significance of these bodies are not known, and a detailed consideration of this phase of the problem is hardly within the scope of the present work.

The position and behavior of the proplastids during cell division have been observed in the living cells. There is apparent no definite sorting out of equal numbers to daughter cells, or more active division of individuals during this period. The proplastids are grouped at opposite ends of the cell during mitosis, and their passive distribution to the daughter cells seems to depend wholly upon their chance positions in the cytoplasm at the time of cell-plate formation.

PLASTID DEVELOPMENT.—The formation of mature functional plastids from minute granular proplastids may readily be followed in the subepidermal cells of the tips of leaf buds forming from the apical meristem. The earlier transitional stages are present in the meristematic cells near the apices of successively older seedling leaves which are growing rapidly, and which are not yet exposed to sunlight. Later stages are found in the leaves which are about to emerge from the surrounding sheath. In passing from the tip of such a leaf toward a point somewhat below the tip a series of stages may be observed. Numerous well developed plastids are present in the mesophyll cells of fully exposed seedling leaves.

In leaf tissue recently formed from the apical meristem the cells contain some proplastids which have increased noticeably in size, and others similar in size and appearance to those found in younger cells (fig. 2). Vacuoles are present in these cells, and the cytoplasm becomes more or less limited to the region surrounding the nucleus and to the periphery of the cell, with connecting strands between. Cytoplasmic streaming, which is commonly active at these stages, carries the proplastids about through the cell, even the largest ones being translocated in this manner.

In somewhat older stages (figs. 3, 4) the largest bodies remain stationary or nearly so, and are not affected by the cytoplasmic streaming. A grouping of proplastids of various sizes is often seen in certain parts of the cell. This most frequently occurs in the parietal portion of the cytoplasm, whereas in certain other cells there may be a grouping of almost all of the proplastids about the nucleus (fig. 51). In the present study these and other modes of grouping have not been correlated with any other phenomenon of cell activity.

Evidence for the division of the developing plastids is not difficult to obtain in the later stages (fig. 5). Cytoplasmic streaming is not so active in such cells, and the proplastids which have reached a size of $2\ \mu$ or over may frequently be seen in the process of division.

A variation in the size of the proplastids which are present in a single cell is markedly characteristic of the tissues studied. In such a cell as that of fig. 6 there is a close intergrading series, from proplastids which are so small as to be barely visible, up to others which have a diameter of $4.5\text{--}5\ \mu$. The largest proplastids of this cell appear faintly green. The presence of these transitional stages within a single cell furnishes convincing proof of the origin of chloroplasts from bodies which at first are scarcely visible, and which one is led to believe may even arise *de novo* from the cytoplasm. In no case has the presence of green color been found to be associated with the proplastids until they have reached approximately one-half of the size and shape of mature functional plastids. The appearance of starch in the developing proplastids, which sometimes occurs, is perhaps associated in some way with chlorophyll formation.

The final stages in the maturing of chloroplasts may be observed in the cells near the tip of a seedling leaf which is about to emerge from the enveloping sheath (figs. 7, 8). Subepidermal cells near the apex contain a small number of partially developed plastids in which chlorophyll is present, and many smaller proplastids of varying size (fig. 7). Farther back from the tip the mesophyll cells contain an increasingly large number of nearly mature plastids, the majority of which are markedly green, and a diminishing number of proplastids (fig. 8). Finally, in the cells of

mesophyll tissue of well developed seedling leaves which are exposed to sunlight the enlarging plastids have attained a diameter of 7-8 μ . In such cells the plastids are closely packed in the parietal layer of cytoplasm, and are intensely green (fig. 9). In these cells, in addition to the larger plastids there are present a certain number of smaller bodies of varying size which cannot be distinguished from the proplastids found in the earlier stages. This fact seems to indicate that such structures represent partially developed plastids and not bodies of a separate category, as some observers have believed. Thus not only meristematic cells, but cells of mature mesophyll tissue as well, contain proplastids in various stages of enlargement, as well as plastids which have attained the maximum size and color.

The condition found in the mesophyll cells of the leaf tissue of a mature plant is very similar to that just described for fully green seedling leaves. In the former the plastids are slightly larger in the exposed portion of the leaf blade, and the number of proplastids is somewhat smaller than in the latter. In portions of mature leaves, however, which are protected from sunlight by other enveloping leaves or leaf sheaths, the development of the proplastids has not progressed as far as in the exposed portion of the leaf, regardless of the fact that the tissue in question is structurally mature. Chlorophyll is almost or entirely absent in such cells. In passing outward toward the green portion of the leaf, the proplastids become progressively larger, and there is a relatively smaller number of more minute primordia. Chlorophyll makes its appearance with the development of the plastids. Figs. 42-46 illustrate the transitional stages found in such a leaf, and the amount of shading used in representing the proplastids indicates the relative intensity of green color to be found in these stages.

There appears to be a close correlation between the development of plastids and the appearance of chlorophyll in the leaf tissue of normal green plants, whether in seedling stages or in the mature plant tissue. Maturing plastids 4-5 μ in diameter are very faintly green, and as they increase in size the intensity of the green color also increases, until the plastids have attained their maximum dimensions, when they are bright green.

EPIDERMAL CELL INCLUSIONS.—In maize the epidermal cells of leaves rarely contain plastids; if present at all they are few in number. Certain very characteristic structures are present, however, which will be discussed only briefly, inasmuch as they seem to have no direct bearing on the problem of chlorophyll development.

The epidermal cells of the meristematic region may be studied without removing them from the remainder of the leaf tissue. Their walls are not heavily cutinized during their early development, and the cells beneath are sufficiently transparent to permit a careful examination of cytoplasmic structures. The cytoplasm is limited to the parietal region of the cell, except for strands which pass through the large central vacuole to the region occupied by the nucleus near the inner wall. The cytoplasm of cells near the tip of embryonic leaves contains minute granules and short rods (fig. 47). These bodies are very numerous, and are carried rapidly about by the flowing cytoplasm, both rods and granules continually changing their positions. In older cells, that is, those farther removed from the tip, the rods are longer and more numerous (fig. 48). In still older cells many elongate filaments are to be found, as well as numerous granules and short rods (fig. 49). Cells have been observed in which even the elongate filaments are actively translocated by the cytoplasm; a single one may thus be observed for some time. This phenomenon furnishes definite proof that the filaments are structurally distinct elements, and not merely lines of flow, or other artifacts due to fixation. The fact that the filaments are longer and more numerous in the older cells than in meristematic cells suggests that they have resulted from an elongation of shorter ones, and that these in turn may have originated from the granular bodies. Convincing proof of their division has not been obtained. A small number of colorless bodies which resemble the partly developed plastids of mesophyll cells are also found in these cells, but mature green plastids are rarely present. In addition to these structures, oil globules are present which may be distinguished by their greater refringence in living cells and by their characteristic reaction to osmic acid. The relation of these structures found in epidermal cells to the proplastids of mesophyll cells will be discussed later.

MENDELIAN WHITE STRAIN

In the "Mendelian white" strain studied there are completely green and completely white plants. Albinism is here inherited as a simple Mendelian recessive. The green plants are entirely like green plants of normal strains, so far as any visible structures are concerned. Microscopic examination of the cells reveals a series of stages in plastid development which is indistinguishable from the series observed in normal green plants already described. In the white (albino) seedlings proplastids are present in the early embryonic stages just as in the green plants (figs. 10-12). In older tissues (figs. 13-16), however, corresponding to similar regions in a green plant (figs. 7-9), there is a striking difference in the behavior of the proplastids. They increase in size very slowly and irregularly, and although the leaf tissue itself continues to grow and differentiate, doubtless at the expense of reserve food stored in the seed, the proplastids do not develop as rapidly, or in as large numbers, as in the tissues in the same stages of differentiation in a green plant. Granules measuring $0.8-1.2\ \mu$ in diameter are found just as in normal green tissue, but development beyond this stage occurs only in a few scattered instances. The few proplastids which do become larger frequently present an abnormal appearance (figs. 14-16). In the living cells they are colorless or nearly so, and may sometimes have a darker irregular mass near the periphery (fig. 15). When fixed according to BENDA's method and stained with haematoxylin, they bear a striking resemblance to small nuclei, and the irregular mass within these bodies bears a striking resemblance to densely staining nucleoli. When the tissues have reached a stage comparable with that at which chlorophyll appears in a normal green plant, there are very few plastids as large as those in which chlorophyll first develops in the green plant, the cell being characterized rather by a large number of proplastids, a condition suggesting retarded development (fig. 16). There also occur irregularly shaped masses, giving the appearance of degenerating plastids which had become partly mature. Figs. 14 and 15 represent the appearance of the cytoplasmic bodies in mesophyll cells occurring between the vascular bundles of the leaf. Fig. 16 shows the condition which is found in a cell nearer the bundle.

Here the proplastids in general are larger and more numerous, and often faintly yellowish green. This appears to indicate that the first steps of chlorophyll elaboration are initiated in these cells.

Although not any of the plastids are normally green in most of the tissue of the leaf blade, there has been found at the extreme tip of the albino seedling leaf a limited region in which the cells contain green chloroplasts (figs. 17-19). In such a region, even within a single cell, are found transitional stages between colorless partially mature plastids and those which are fully green (fig. 18). Chlorophyll, therefore, is not entirely absent from the albino seedlings of the "Mendelian white" strain. In these albino seedlings, however, there is never developed a sufficient amount of chlorophyll to enable the plant to live beyond the seedling stage. Microscopic examination shows clearly that the initial structural basis for plastid development is present, chloroplasts which are normal in appearance being actually found in some cases. This latter condition never becomes general throughout the plant. It therefore seems clear that in this case failure of the plant to become green is not to be explained as the result of an absence of plastids or plastid primordia.

MENDELIAN VIRESCENT

A third category is "Mendelian virescent." In this strain the affected plants in the young seedling stage resemble those of the "Mendelian white" strain. The seedling leaf is at first white, but later nearly the entire leaf becomes green, the color deepening rapidly. Later formed seedling leaves are somewhat greener in the early stages, while leaves formed toward maturity are entirely green from the start.

In the virescent plants the early stages in the development of the plastids are similar in all respects to the corresponding stages in a normal green plant. So far as it is possible to tell, the number, size, and development of the proplastids is identical in the two cases (figs. 20-23). In the tips of the young leaves, as they increase in size and become exposed to sunlight, the growth of the proplastids and the development of chlorophyll proceed as in green plants (figs. 24-26, 32). In the main portion of the leaf blade, on the

other hand, the mesophyll tissue fails to become green as rapidly as the tissue near the tip, and the cells between the bundles are found to contain only partly developed proplastids (fig. 27). Cells nearer the vascular bundles contain larger plastids with more chlorophyll (fig. 28), while in cells adjoining the bundles the plastids have reached nearly the maximum size and color (fig. 29). In somewhat older leaf tissue which is gradually becoming green there is a corresponding increase in the size of the proplastids (figs. 30-32), and the resulting plastids eventually become fully green (fig. 32). Here, then, is a case in which the usual formation of chloroplasts with their pigment seems to be merely delayed, but, unlike the "Mendelian white" seedlings, development continues until the whole plant becomes fully green.

MATERNAL INHERITANCE STRAIN

In this strain there are plants of three kinds. Some are entirely green and others are uniformly yellowish green; a third class is made up of plants which show longitudinal stripes of the two colors. The number and character of such stripes are extremely variable. Some plants are distinguishable from normal green plants only by the presence of one or two stripes, which may be of any width, and which may be of a color only slightly lighter than normal green. On the other hand, the appearance of some plants is rendered most striking by a large number of stripes, which, although their yellowish green color varies in depth to a certain degree, are never white. Frequently a plant may be divided sharply and almost equally throughout its entire length into green and yellowish green halves. Again, plants are observed which are completely yellowish green, except for one or two fully green stripes. The light stripes, whatever the intensity of their color, are not always continuous from the base to the tip of the leaf. In such cases they may end more or less abruptly at any point in the leaf, or may begin at any point above the base and terminate anywhere between this point and the tip.

CYTOLOGICAL FEATURES.—With regard to the green plants, careful cytological examination has failed to reveal any differences between their cells and those of green plants of unaffected strains.

The cells of the yellowish green plants show a series of stages in the development of chloroplasts from minute proplastids which are similar in all respects to the stages in the green plants, with the exception of the maximum size and depth of color attained. The proplastids increase in size until many of them have become well differentiated plastids with a diameter of $4.0-5.5\ \mu$ (figs. 33-41), whereas the plastids in normal green plants have a diameter of $7-8\ \mu$; very exceptionally the latter size may be attained (fig. 41). The plastids in the pale areas of the seedling leaves are fewer in number, and the proplastids are more numerous than in the corresponding cells of a green plant (fig. 39). A study of the living cells shows a variable behavior as regards the elaboration of chlorophyll in the young seedling leaves. In the mesophyll cells most of the plastids become faintly green as soon as they become partially mature (fig. 39), while in cells near the vascular bundles plastids are often observed in which the color has become nearly as deep as in a normal mature chloroplast (fig. 41). In cells somewhat removed from the bundles all gradations between the lighter and darker shades are seen (fig. 40). In addition to the uniformly colored plastids, there are others which show an uneven outline and an irregular distribution of the pigment within them. For example, the color is often confined to one or two regions within the plastid, the limits of these regions in some cases being clearly marked and in others very vague (fig. 39). Such appearances strongly suggest degeneration or other disturbances of a serious nature. Not only young cells, but cells which are completely matured, contain proplastids in various stages of enlargement.

The plastids are much more fully developed in the yellowish green plants than in the albino seedlings of the "Mendelian white" strain, but a sufficient amount of chlorophyll for continued growth fails to be developed, and the plants die in the seedling stage. They may become slightly larger, however, than the white seedlings of the Mendelian strain. In the yellowish green plants there is little correlation between the degree of development reached by the plastids and the amount of chlorophyll elaborated. There are abundant well differentiated plastids present but only a rela-

tively small amount of chlorophyll. The failure of the plants to become green cannot be ascribed to a failure of the plastids to develop, but rather to an absence of a sufficient amount of chlorophyll.

The striped plants of this strain possess varying amounts of green and yellowish green tissue, as already pointed out. To the naked eye the boundary between the green and yellowish green areas appears to be very sharp, but when viewed with the microscope there is seen a region of transition one to several cells in width, in which are found plastids showing many intermediate sizes and depths of color, even within a single cell. Although the transition regions may vary in width, careful search has so far never failed to reveal cells which are in some degree intermediate in character. In some cases there is a single transitional cell which may contain plastids of many shades of green, whereas in other cases there may be a series of several transitional cells in each of which all or nearly all of the plastids are of one intermediate shade. Fig. 50 illustrates the condition which is most frequently found to occur in transitional regions. The cells on the left are characteristic of yellowish green tissue, those on the right are typical of green tissue, the ones between contain plastids which vary greatly, both in size and in intensity of green pigment. In the light of such facts the inapplicability of hypotheses involving a simple sorting out of plastids of two completely distinct types by successive cell divisions is clearly evident, so far as the color types in the strain of maize under consideration are concerned. To this point we shall return.

Intra-vitam stains and the use of fixing and staining reactions commonly employed have as yet given no evidence that the class of cell elements described comprise bodies of more than one kind. It is hoped that further studies may contribute something to this phase of the problem.

Summarizing, all of the plant types examined have been found to be the same as regards the cytoplasmic inclusions of their meristematic cells. Minute proplastids of the same size, shape, and general appearance have been observed in the living cells of all the chlorophyll types of maize studied. Intra-vitam stains and the fixing and staining reactions which have been used by other

workers for the study of cytoplasmic structures have given no evidence for the assumption that the group of cell elements under investigation consists of bodies belonging to more than one class. The observed differences between the various types of plants lie in the subsequent behavior of the proplastids with reference to the development of chloroplasts. From a structural standpoint the cells of all the plant types appear to be initially alike. The differences which later appear seem rather to be dependent upon the relative amount of chlorophyll developed in organs, the primordia of which are present in all cells. It is probable, therefore, that the ultimate explanation of unusual types of behavior may involve functional rather than structural differences.

Discussion

BEARING OF RESULTS ON PLASTID INHERITANCE.—The hypotheses which have been advanced to explain plastid inheritance have been based chiefly on the breeding behavior of the plants. The question remains as to how far these hypotheses will be substantiated by cytological observations. For the most part such hypotheses involve the assumption that plastids are permanent cell organs, arising only by a division of others of their kind, either while they are in the form of minute primordia or after they have reached the mature stage. The fact that proplastid-like bodies are present in the early embryonic stages, and the well substantiated evidence that mature plastids arise from bodies which cannot be distinguished from these elements, however, do not seem to constitute sufficient proof of the theory that plastids are cell organs having an unbroken continuity through all stages of the life cycle. Observations suggest that plastids may also differentiate anew at certain times in the life of the cell, which is obviously of the highest importance in connection with the problem in hand, and will be fully discussed later.

The frequent occurrence of chlorophyll abnormalities has furnished abundant material for a study of plastid inheritance. Many cases have been reported, and the breeding behavior carefully studied. The inheritance of some of the known cases is clearly Mendelian, but other types are clearly non-Mendelian. Thus the

known cases of plastid inheritance do not appear to be in the same category, and as yet have not been explained by a common well substantiated hypothesis.

Examples of chlorophyll characters which are inherited as Mendelian recessives have been described by numerous workers. Among the researches dealing with such characters may be mentioned those of EMERSON and LINDSTROM in maize, and those of BAUR, KIESSLING, TJEBBES and KOOIMAN, MIYAZAWA, CORRENS, and ALLARD on a number of other plants. The hypothesis held to account for the transmission of these characters is the same as that upon which the behavior of other Mendelian characters has been explained. Although the character in question is expressed in the plastids, the cell activities which result in the appearance of the character seem to be under the control of nuclear factors. If the character is thus under the control of a nuclear factor, it is nevertheless of importance to determine whether plastids are present in colorless cells as well as in normal ones; and also to determine to what extent the plastid is a permanent cell organ multiplying by division, especially if it is desired to gain insight into the process through which the character is brought to expression.

MILES (47), in a cytological investigation of albino seedlings in maize, reported that in the pure white plants no plastids could be differentiated. This does not at all correspond with the findings of the writer. Partially matured plastids are present in all of the mesophyll tissue of the plant, and in certain regions a limited number of mature functional plastids are present in some cells. Plastids, therefore, are not entirely absent, but their development is permanently retarded in the white seedlings. In the virescent plants the normal development of the plastids is interfered with only in the seedling stage.

Other chlorophyll types in maize which are Mendelian appear to be normal in the seedling stage, but the character appears in the plant as it approaches maturity. In fact there are known in maize markedly different chlorophyll types, and it seems certain that additional ones will be described. These cases are of interest cytologically in that apparently there is a wide variation in the condition of the plastids in the affected plants.

The cases in which the inheritance of plastid characters is non-Mendelian present a very complex problem. Added significance is here attached to the plastids, and workers are led to assume that the plastids themselves in the main are responsible for the inheritance of the characters which are manifested in them. As in the case of the Mendelian plastid characters, this assumption involves the question of the origin and permanence of the plastids, to be discussed later. The cases of non-Mendelian inheritance of chlorophyll variation have been classified as (1) maternal and (2) biparental.

Attention has already been called in the introduction to the well known cases of maternal inheritance reported by BAUR, CORRENS, and GREGORY, as well as to that found in maize by ANDERSON, whose results have not yet been published. CORRENS assumes that in *Mirabilis* the absence of chlorophyll is due to a cytoplasmic disease which in some way affects the plastids. Two kinds of plastids are said to be present, green and colorless ones, whose segregation during the divisions of the somatic cells is assumed to explain the presence of "checkered" leaves, as well as the complete absence of color in entire leaves and branches. BAUR offers a somewhat different explanation for similar cases. He believes that there are two kinds of plastids, diseased and normal ones, present in the mature leaf tissue, both being permanent cell organs with a definite individuality. The primordia of the two kinds of plastids are supposed to be transmitted from one generation to the next through the cytoplasm of the egg. A somatic segregation of these primordia to different cells during the growth of the plant accounts for the green and white areas of the mature plant. The hypothetical nature of this explanation is to be admitted, inasmuch as different kinds of primordia have not been demonstrated in these plants, from which it follows that the postulated segregation has not been observed. An examination of the mesophyll cells of plants produced by the maternal inheritance strain in maize fails to lend support to either of these theories. The development of the plastids from granular proplastids has been traced carefully in the living cells, and the condition of the plastids in the leaf tissue of the seedlings and mature plants has been

studied thoroughly. A variation, not only in the size of the plastids, but in the amount of color pigment as well, is characteristic of all the plant types.

Two distinct kinds of plastids, normal (green) and chlorophyll-less ones do not occur in the strains of maize studied; the green and colorless plastids observed in these strains do not represent distinct categories at all. The yellowish green plants of the maternal inheritance strain produce some plastids which have a diameter equal to that of the plastids present in normal green plants, and the intensity of the green color may equal that of green plants. In general, however, the plastids are slightly smaller and paler in color. The striped yellowish green and green plants show no sharp segregation of two distinct kinds of plastids. Cells on the border line between the two regions contain plastids of varying sizes and intensities of color. A single cell in such a region often contains plastids showing all degrees of variation found in either yellowish green or green areas. Such intermediate conditions are prevalent in the transition region between the two kinds of tissue, and at no time can there be found two distinct kinds of plastids, either in different cells or in the same cell. GREGORY made a study of the breeding behavior of a strain of *Primula sinensis* which produced chlorotic plants. The experimental results showed the character to be maternal in its inheritance. In chlorotic cells the plastids were shown to be pale yellow and smaller than in cells of the green tissue, but in any individual cell of mature tissue the plastids were found to be all alike. In young actively growing cells, however, different kinds of plastids occur in the same cell, which are similar to the two kinds found in the green and chlorotic cells of the mature leaf. GREGORY uses this as evidence that both kinds occur together in embryonic tissue, and later become segregated to different regions of the plant, and concludes that the abnormality is localized in the plastids. There is, however, another interpretation to be placed on the variation in the size and color of the plastids present in the growing cells of the leaf. In maize there can be no doubt that this condition is due to the presence of different stages in the development of plastids of one kind. The association of plastids showing a great variation in

size and color in the same cell is characteristic of embryonic cells in normal green plants. Since it was in such cells that GREGORY reported the presence of the two supposedly different kinds of plastids, this interpretation seems to be a more plausible one for his observations. Convincing cytological evidence of the occurrence of two distinct kinds of plastids and their segregation to different regions of the plant is at present lacking. In view of the condition observed in maize, it is clearly evident that visible structural differences in meristematic cells cannot be held to account for the inheritance and development of chlorophyll patterns.

All of the plant types, both the Mendelian and maternal, have the same initial cell structure, so far as observations have gone. It has not been possible to distinguish different kinds of proplastids in the youngest cells examined, that is, those of the promeristematic tissue of very young stems and the meristematic regions of embryonic leaves. The actually observed differences lie in the subsequent behavior of the proplastids with reference to the evolution of plastids.

Whether the proplastids arise by division or *de novo*, they are found to be present in the meristems of all of the chlorophyll types. The fertilized eggs of the different strains have not been examined, but if they differ as regards their visible cytoplasmic inclusions, corresponding differences would certainly be expected in the undifferentiated embryonic tissues of these strains; but careful examination of such tissues has failed to reveal any differences. As already stated, therefore, the differences in such types seem to be due to different modes of subsequent behavior on the part of proplastids in different plants and in different regions of the same plant, rather than to initial proplastid differences in the fertilized eggs of the different types. This view is supported by the occurrence of striped plants in the maternal inheritance strain, and also in the Mendelian strains which are not here reported.

It is necessary to account for different kinds of behavior on the part of proplastids which are, so far as microscopic examination shows, initially alike. Since visible bodies of more than one kind cannot be seen segregating, it might be suggested that there is an invisible structural difference in the cytoplasm of the different

plant types, or even in different parts of the same plant (striped). Thus the difference between the green and white plants in both the maternal and Mendelian categories depends upon an invisible differentiation process in the cytoplasm of the different cells, which influences the course which the proplastids take in their development, and the degree of development reached. One is then led to inquire at what stage in the life cycle this differentiation, which may be regarded as a physiological process, occurs. In the case of the maternal types, the irregular distribution of the green and white plant-producing seeds on the ear suggests that it may occur in the formation of the egg, or even earlier in the development of the ear, because of the frequent presence of irregular patches of affected seeds. The occurrence of the differentiation at some stage in embryogeny would account for the striped plants, in early division stages for the plants with large areas of the pale green tissue, and in the later stages for the plants having smaller amounts of such tissue. In the Mendelian types differentiation which is probably of a different kind must occur at sporogenesis, like any other Mendelian character. The two types of behavior, the Mendelian and maternal, are not in the same category, and as yet cannot be explained by a common well substantiated hypothesis. It is hoped that further studies of cytoplasmic structure will furnish evidence of value in the solution of the problem, and show more clearly what phases of the problem are to be definitely assigned to the physiologist.

RELATION OF PROPLASTID TO CHONDRIOSOMES.—One of the most outstanding questions arising from the present study is that regarding the relation which may exist between the proplastid of the foregoing description and the minute cell elements which other investigators have described under the name of chondriosomes, mitochondria, etc. There is great confusion at the present time concerning the nature and significance of these elements. It is plain from a survey of the very extensive literature dealing with the cell elements of this general class that the opinions of the most competent observers are in conflict on many important points. It is probable that cell inclusions of many different kinds may have been designated by the term chondriosomes, and whether

any particular body falls under this class depends upon the definition employed.

The present study has shown beyond a doubt that plastids develop from minute primordia in the different types of maize under consideration. What has so far been determined regarding the nature of these primordia and the relation which they may bear to chondriosomes must be regarded as insufficient to warrant an extended critical review of this question. It is planned to continue the investigation of this point. Nevertheless a brief comparison with the observations of other investigators who have devoted special attention to the chondriosomes may contribute something toward a solution of this puzzling problem.

The development of plastids from granular primordia has been observed by many workers, but there is a wide diversity of opinion as to the relation of the primordia to other cytoplasmic inclusions. One group of workers maintains that plastids arise from chondriosomes (mitochondria) which are permanent cell organs, and which correspond to similar bodies described in animal cells (MAXIMOW, PENSA, LEWITSKI (41), GUILLIERMOND, EMBERGER). GUILLIERMOND (24-34), as a result of many researches on plant cells, believes that plastids arise from rodlike mitochondria (chondriokonts) and differ from the latter only in size and to a certain extent in chemical constitution. TWISS (56) reports that in fixed and stained root tip cells of *Zea Mays* an unbroken series from globular, ellipsoid, or short rod-shaped mitochondria to mature plastids can be traced from the embryonic region backward, and concludes that mitochondria are normal constituents of the cytoplasm. He believes, however, that the evidence for the division of the mitochondria, as well as that for their function in heredity, is inadequate. EMBERGER (18) reported that there are two kinds of bodies (young plastids and mitochondria) present in the cells of certain plants, which differ slightly in size and staining intensity; both are to be classed under the general term mitochondria. Other workers hold that the plastids do not come from chondriosomes, but from bodies distinct from them (48, 50, 52, 53, 54). A third group of workers (TISCHLER, VON DERSCHAU, etc.) contend that the plastids are ultimately of nuclear origin, since they arise from chromatic

bodies extruded from the nucleus. Still others question the relationship between plastids and chondriosomes. HARPER (35) states as follows:

None of the evidence so far adduced as to the specific genetic relationship between chondriosomes and plant plastids is in any way adequate that in certain cells the plastids can be recognized as very small cytoplasmic bodies with no starch in them was adequately established by SCHIMPER, but that the plastid bodies necessarily and regularly arise from the chondriosomes it seems to me is by no means proved by such crude and diagrammatic figures and seriations as those so far presented.

Views similar to this have been held by many other workers (LUNDEGARDH 46, LÖWSCHIN 44, 45, etc.).

From this brief review it is evident that there are, at present, many conflicting views in regard to the class of cell inclusions known as chondriosomes, and the relationship which exists between plastids in their initial stages and these bodies. It is furthermore apparent that this diversity of opinion is in some measure a consequence of the lack of agreement as to what constitutes permanent cytoplasmic organs, and as to what bodies should be included in the class "chondriosomes." The problem has been complicated further by the fact that certain observers have used a great variety of fixatives, which in some cases appear to have preserved the bodies in question with relatively little alteration, while in other cases artifacts have been produced which have led to various misconceptions.

No reader of chondriosome literature can fail to recognize the fact that much of the obscurity which surrounds the subject is due to the lack of uniformity in the application by various workers of the term chondriosome. By some the term has been applied very widely to a considerable variety of minute cell inclusions, some of which are seen to develop into plastids. Others are inclined to make a distinction between chondriosomes on the one hand and the primordia of plastids on the other. Still others further restrict the application of the term chondriosome, using it only with reference to a concrete class of cell inclusions which can be shown to have a particular histochemical constitution. Thus COWDRY (10) defines chondriosomes primarily as "substances which occur in the form of granules, rods, or filaments in almost all

living cells, which react positively to Janus green, and which, by their solubilities and staining reactions, resemble phospholipins and, to a lesser extent, albumins."

Noteworthy in this connection are the statements made by MOTTIER (50) as a result of his studies on the origin of plastids. In his first contribution on this subject leucoplasts and chloroplasts were shown to be derived from minute "plastid primordia." Other minute bodies present in all cells but not developing into plastids were referred to as chondriosomes. In a more recent paper (51), in which the development of protein bodies (aleurone grains) from minute primordia is reported in the endosperm cells of *Zea Mays* and other plants, emphasis is no longer placed upon this distinction between primordia and chondriosomes. It is stated that the latter, because of their obscure rôle, were previously called chondriosomes mainly for convenience. In view of the fact that the terms mitochondria and chondriosome have been so loosely applied to plastid primordia and many other cytoplasmic inclusions as well, MOTTIER does not use these terms in his most recent description, and even suggests that much ambiguity might be avoided by dropping the terms entirely.

The present writer has also felt that much confusion might result from the use of the term chondriosome in his description. In the material studied there appears to be but one class of cytoplasmic granules, as already stated, these granules representing the early stages in the development of plastids. In order to avoid any implication as to the ultimate nature of these bodies and their relation to other cytoplasmic inclusions described by various workers, they have been designated in the present paper by the term proplastid. Whether or not the proplastid is a chondriosome is a question which can be answered only when the histochemical nature and developmental history of cytoplasmic inclusions have become better known, and when a definite and uniform terminology has been settled upon. The proplastids can be readily observed in the living cells. They are clearly visible in the cells of the promeristematic tissue of young stems and embryonic leaves, and their activities in these cells and their subsequent behavior in older cells have been closely followed through all stages of their develop-

ment. Although they can be so studied in the living cells, it is nevertheless necessary to employ methods which will not only permit one to view the living cell for a moment in the "living" condition, but which will allow continued observation of the actively functioning cell over a considerable period of time, thus avoiding alteration in the cytoplasm which may be brought about very quickly when the living tissue is improperly handled.

The proplastids appear in their initial stages as refringent globules of varying size, always sharply distinct from the ground substance of the cytoplasm. They are constantly changing their position in the embryonic cells, being often carried about rapidly by the streaming cytoplasm. This phenomenon makes it appear certain that even the most minute of the proplastids are special structures distinct from the principal mass of cytoplasm in which they lie. Particular attention has been devoted to these minute proplastids which lie just within the lower limit of the range of visibility. They have been examined with lenses of very high resolving power (Zeiss apochromatic objective, 2 mm. N.A. 1.40 and compensating ocular 6), and their activity has been repeatedly watched very closely. Very important questions concerning the ultimate nature and significance of proplastids are here involved, and will be discussed in another connection later. The subsequent enlargement of the proplastids to form chloroplasts can be traced step by step throughout their course of development, abundant material being readily obtainable from young seedlings.

Although comparatively few investigators have based their interpretations mainly on the study of living cells, they agree with the present writer in reporting the presence of such minute bodies in the living meristematic cell. In older cells, however, many phenomena have been described which the present writer has not observed, although this may be due in part to the fact that this study has been limited largely to mesophyll cells. The few observations made on the cells of other tissues indicate that additional types of cell elements and modes of behavior may be present there. For example, conditions similar to those figured by GUILLIERMOND in epidermal cells of flower petals and other floral organs have been seen in the epidermal cells of maize, but not in the mesophyll cells.

Intra-vitam stains, a number of which have been employed, have unexpectedly failed to give a definite reaction to the proplastids. Such stains as Janus green B, violet of Dahlia, neutral red, etc., have given uncertain results. In some cases the proplastids have appeared to be faintly stained by Janus green B, but in no case has a strong staining reaction been observed, neither has it been found possible to differentiate different kinds of minute bodies in the cytoplasm of the cell. COWDRY (11) reported that plant mitochondria are stained with Janus green B, but that it is much more difficult to obtain a good stain in plant cells than in animal cells because of their well developed cellulose walls. A similar conclusion has been reached by other plant workers.

Chondriosomes in both plant and animal cells were first observed in fixed and stained preparations, and the lack of uniformity in the results of early workers may have been largely due to the use of different fixatives and alterations resulting from poor fixation, as has been pointed out above. The special methods of fixation and staining commonly used in a study of the cytoplasm have been fully described in recent literature (COWDRY 11, GUILLIERMOND 31, KINGSBURY 39). In general chondriosomes have been found to be preserved by potassium bichromate, chromic acid, neutral formalin, and osmic acid, and these are the chief ingredients of the fixatives used for their study. Such substances as alcohol, ether, chloroform, and acetic acid dissolve the chondriosomes or produce profound changes in their shape, etc.

The methods of fixation ordinarily employed often appear to preserve quite faithfully the structures in question, but direct observation on the living cell is unquestionably of far greater value, since it obviates the possibility of misinterpretation to a very large extent. Furthermore, in tissues which are favorable for a study of the living cells the cytoplasmic inclusions are sharply delimited, and actually are more easily observed than in fixed and stained cells. Conflicting results have been obtained by those who have attempted to demonstrate the existence of different kinds of cytoplasmic bodies by the use of various fixing and staining methods. The use of such methods in this study has failed to furnish convincing evidence for the presence of more than one type of proplastid in the initial stages.

Chondriosomes of widely different shapes have been observed, the commonest forms being the granular mitochondria and the rod or thread-shaped chondriokonts. These and other types are not looked upon as belonging to distinct classes by plant cytologists, but rather as transitional stages between granular elements and structures such as chloroplasts, chromoplasts, elaioplasts, etc., concerned in the elaborative functions of the cell. In animal cells the chondriosomes have been seen to undergo marked changes in shape (LEWIS and LEWIS 40), but it may be that such changes are more gradual in plants. The proplastids in maize are of a single uniform shape in the mesophyll cells, being granular in the early stages and spherical or ovoid in later ones. Rodlike elements are present in the epidermal cells, but not in the cells associated with chloroplast formation. This variation in the size, and the different reactions to certain methods of fixation and staining have been used by workers as evidence for the existence of different kinds of elements which can be distinguished in embryonic cells and which perform certain definite functions. Prominent workers maintain that plastid primordia can be distinguished from mitochondria at all stages in their development (RUDOLPH, MOTTIER, DANGEARD). This conclusion is based chiefly on size differences and on slight variations in fixing and staining reactions. This contention has been supported by the statement that unaltered mitochondria are present with the chloroplasts in mature cells, the inference being that all the plastid primordia have developed into plastids, while the mitochondria remained unchanged. The statements of MOTTIER already cited are noteworthy in this connection. The present study has shown that in maize these small bodies in the mature cells are without doubt to be regarded as plastids which are for the most part in a very retarded state of development. A sufficient number of intermediate stages between the smallest ones and mature plastids are present to make it appear certain that these bodies do not represent a distinct category unlike the proplastids of meristematic cells. This question of the possible existence of more than one type of initial granule has received critical attention throughout the present study, and inasmuch as the origin of the chloroplasts can be traced backward through

successively smaller stages to proplastids which lie just within the lower limit of visibility, there appears to be no justification for the view that more than one kind of initial granule is present in the meristematic cell. It is only through a study of these minute initial stages that many of the principal questions involving chondriosomes and plastids can be answered. These earliest stages seem to have received but scant attention from previous workers. It is to be emphasized that the evidence at hand leads directly to the conclusion that the various structures observed in later stages (green and white plastids) have arisen by a process of differentiation from bodies of one kind, rather than from bodies initially unlike. The cause of such a difference in behavior on the part of the primordia can only be conjectured.

INDIVIDUALITY OF PLASTIDS.—The proplastids have been found to be a constant feature of cytoplasmic organization in maize, and the question arises as to the origin of these bodies. The definiteness of their behavior in relation to the evolution of chloroplasts indicates that they are at least concerned in the elaboration of certain products of cell activity. Whether or not they may also be found to be associated with other vital functions remains as a problem for further research.

Plastid primordia have been considered by previous workers as essential constituents of the cytoplasm which retain their individuality and persist throughout the life cycle (FORENBACHER 21, PENSA, CAVERS 5, GUILLIERMOND, MOTTIER, EMBERGER). The evidence from primitive plants (ALLEN, SAPEHIN, SCHERRER), in which well developed plastids are present throughout the life cycle, may be considered as evidence favoring this view. The occurrence of division stages in partially developed chloroplasts suggests also that the smaller bodies may divide, but my observations indicate rather that such an assumption is hardly warranted. Appearances have frequently been found which suggest division stages, but definite proof of division is very difficult to obtain. The fact that a frequent association and subsequent separation of the proplastids occur in embryonic cells, as herein described, renders it unsafe to draw conclusions on this point. Even if it were shown that these minute bodies multiply by

division only, it would still remain to be shown whether or not the same would hold true for stages before the bodies have become large enough to be seen. The writer has repeatedly satisfied himself that the series of stages observed actually grades off to the lower limit of visibility.

In view of the uncertainty regarding the mode of plastid origin in young meristematic cells, it seems probable that the question of the genetic continuity of plastids would not be answered with finality by a study of the gametes by methods at our command. In the present research the gametes have not been observed. If it were found that the fertilized egg contains no visible proplastids, the presumption would be strong in favor of the *de novo* origin of these bodies in embryonic cells. It is conceivable, however, that proplastids too minute to be observed might nevertheless be present and be multiplying only by division. On the other hand, if the fertilized eggs were found to contain proplastids, the mode of their origin and multiplication would probably be no easier to determine than in older meristematic cells which have been studied and described. It therefore seems that the impossibility of determining the mode of proplastid origin in meristematic cells requires that the question of the continuity of plastids shall remain an open one, regardless of any condition which one might expect to observe in the gametes. In view of these facts, explanation of plastid behavior which are based on the assumption of a complete individuality on the part of these organs are unsound. The only other alternative is that of *de novo* origin. The conception of the cytoplasm as a substance in which certain processes become localized, with the accompanying new differentiation of regions which are cell organs, has been furnished with a clear statement by HARPER (35), as follows:

What seems to me the most important advance in our knowledge of cell architecture has been in the direction of the recognition of localized spatially differentiated regions of the cell body in which certain processes occur the plastid is to be regarded as a region of the protoplasmic complex rather than a differentiated and definitely delimited body cytologically the chloroplast is perhaps little more than an area of the cytoplasm impregnated or infiltrated with chlorophyll.

The possibility of the *de novo* origin of cell organs is one which should be more generally recognized. With regard to the plastids it must be admitted that the evidence so far obtained by cytologists does not permit a definite decision in favor of either the *de novo* or the individuality theory. The exact manner in which plastids originate in the cell is obviously of the greatest importance to those who are searching for the explanation of the behavior of inherited characters which manifest themselves in these organs. If plastids are not passed on as permanent individuals, some other explanation must be offered for their repeated appearance and regular behavior in successive generations.

Summary

1. All the chlorophyll types examined were found to contain the same initial cell structure, minute "proplastids" of the same size and general appearance being present in every type.

2. In normal green plants the proplastid first appears in the cell as a minute granule at the limit of visibility, gradually enlarging and developing chlorophyll until it becomes a mature chloroplast. In plants of the other chlorophyll types studied (Mendelian white, Mendelian virescent, and the maternal inheritance strain) the unusual characters of the plants are due to the failure of the proplastids initially present to develop into plastids with the normal size, or color, or both.

3. The green and colorless plastids found in different plants or in different portions of the same plant do not represent two fundamentally distinct types, but are rather to be regarded as the end members of a continuous series which comprises also all intermediate conditions. No cytological evidence was found favoring the view that the primordia from which the variously developed plastids arise are of more than one kind.

4. Partially developed and fully matured plastids may be seen multiplying by division, but when first visible the proplastid is so minute that it is impossible to determine the mode of its origin. The division of partially mature and mature plastids emphasizes the fact that they have a distinct individuality at such stages;

but in view of the obscurity which surrounds the origin of the minute primordia from which the plastids first appearing in the embryonic cells arise, the question regarding the extent to which the plastids are to be considered permanent cell organs with an unbroken genetic continuity throughout the life cycle must remain an open one.

5. In the case of those strains in which the inherited characters are transmitted according to Mendelian rules, it is inferred that the behavior of the proplastid is at least in part under the control of the nuclear mechanism. In those strains in which the inheritance of the unusual characters is non-Mendelian (maternal strains), it is very probable that an explanation of another kind will be found necessary.

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EXPLANATION OF PLATES XI-XVI

All figures were drawn at the level of the table with the aid of an Abbé camera lucida under a Spencer objective, 1.8 mm., N.A. 1.25, with compensating ocular 18. They have been reduced approximately one-third and show a magnification of about 2250 diameters. Observations of critical stages were made with a Zeiss apochromatic objective, 2 mm., N.A. 1.40, with compensating ocular 6.

PLATE XI

Normal green plant

Except where noted, drawings are of living cells of subepidermal tissue from mesophyll region of leaf, or of meristematic cells which will later become part of mesophyll tissue.

FIG. 1.—From promeristematic region of germinating seed.

FIG. 2.—From tip of first leaf bud formed from apex of stem; median section.

FIGS. 3-6.—Developmental stages from meristematic tips of successively older embryonic leaves; figs. 3-5, median sections; fig. 6, section through cytoplasmic layer just beneath cell wall; largest proplastids in fig. 6 faintly green.

FIGS. 7, 8.—From 16 mm. embryonic leaf just before it emerges from sheath, showing cytoplasmic layer beneath cell wall in face view.

FIG. 7.—Cell near tip, green color appearing in proplastids.

FIG. 8.—Cell somewhat farther back from tip, proplastids more fully developed.

FIG. 9.—From mesophyll tissue of fully developed seedling leaf, plastids bright green.

PLATE XII

Mendelian white seedling

Amount of green color present in chloroplasts after they have reached a size of $2.5\ \mu$ indicated by depth of shading; stages represented in this series taken from a 3-inch seedling about six days after germination.

FIGS. 10-12.—Successively older stages in development of proplastids in undifferentiated meristematic tissue; cells in median section; fig. 10, promeristematic cell from stem tip; fig. 11, from tip of leaf bud recently formed from apex of stem; fig. 12, from tip of 18 mm. embryonic leaf.

FIGS. 13-16.—From different regions of 35 mm. leaf just before emerging from enveloping leaves; fig. 13, undifferentiated cell near tip (note abnormal appearance of some of proplastids); irregular shape and presence of darker regions within proplastids characteristic of certain ones; figs. 14, 15, cells 3-5 mm. from tip; fig. 14, green color entirely absent in all proplastids; fig. 15, faint greenish color in one proplastid; others show conditions suggesting degeneration; fig. 16, typical mesophyll cell of seedling leaf; slight trace of yellowish green color in some of proplastids; others almost colorless or containing irregular opaque masses.

FIGS. 17-19.—Cells from tip of unfolding seedling leaf.

FIG. 17.—Undifferentiated cell at tip of leaf; largest proplastids faintly green.

FIG. 18.—Cell 3 mm. from tip; larger proplastids (plastids) clearly green.

FIG. 19.—Mesophyll cell 4.5 mm. from tip; plastids bright green.

PLATE XIII

Mendelian virescent

Depth of shading indicates intensity of green color in later stages of plastid development; material of same age as in the case of Mendelian white.

FIGS. 20-23.—From promeristematic and embryonic leaf tissue corresponding to similar early stages in green plant.

FIGS. 24-26, 32.—From meristematic undifferentiated regions of successively older leaf tips; figs. 24-26, before exposure to sunlight; fig. 32, after exposure to sunlight.

FIGS. 27-29.—Cells from pale green area of seedling leaf.

FIG. 27.—Mesophyll cell between vascular bundles.

FIG. 28.—Mesophyll cell nearer vascular bundle.

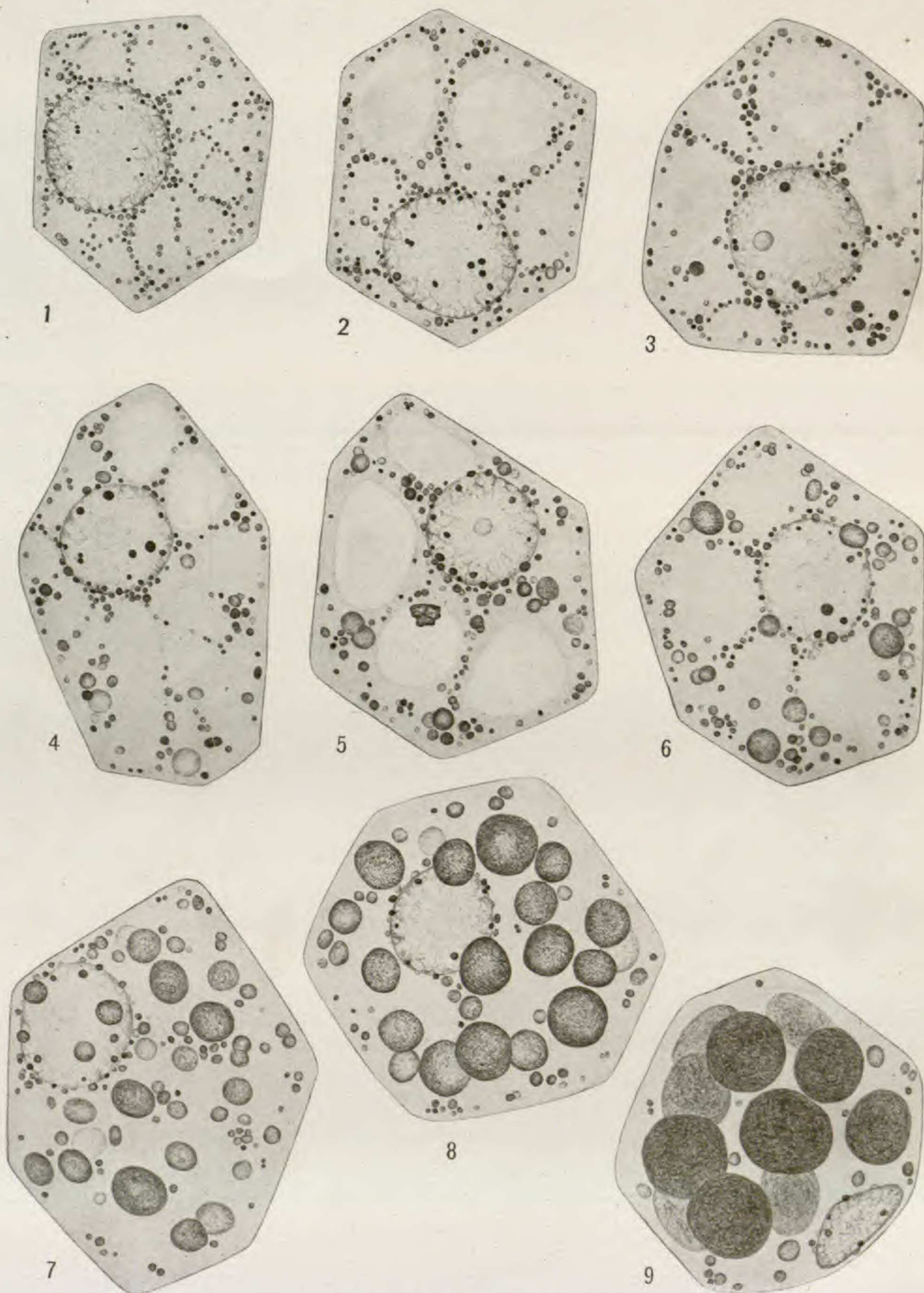
FIG. 29.—Cell lying next to vascular bundle.

FIGS. 30-32.—From transition region between pale green and green areas of seedling leaf.

FIG. 30.—Mesophyll cell from slightly green region.

FIG. 31.—From deeper green region.

FIG. 32.—Cell from apex of green leaf.



RANDOLPH on MAIZE